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METHOD FOR GROWING BASIDIOMYCETES
[PROCEDIMIENTO PARA EL CULTIVO DE BASIDIOMICETES]

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DESCRIPTION

As its title indicates, the invention concerns some improvements in the method of growing fungi of the family of basidiomycetes and, especially and preferably, improvements in the method described in patent No. 8701468 of the same inventor - applicant.

The descriptive part of the above-mentioned patent stated the difficulties encountered in traditional culturing methods and the precautions that must be taken in order to avoid contamination by fungi or bacteria that easily develop in many environments for many reasons.

Logically the basidiospores of Agaricus bisporus germinate and a mycelium is obtained from them on a sterile culture medium that constitutes the starting point for the seeding or culturing of the product that has to be performed under the conditions and with the precautions indicated in the text of the previous patent.

This patent offers a method for growing basidiomycetes based on a special culture bed, composed of the residues of oily fruit, and specifically of olive pomace.

The method involves the use of the residual product of crushing (olive pomace), treating it in accordance with a process that involves maintaining certain moisture levels, in appropriate premises and environments, and seeding it by means of an appropriately studied and approved method, media, and steps.

The previous method likewise involves successive steps in which the culture media, the forms of making it, the times, temperatures,

etc., are developed.

However, this method, just as the growing procedures known whatever the method used and the culture bed used in them, require the incorporation of seeding material, that is, the logical or recommended proportions of basidiomycete mycelia obtained in pure sterile preliminary cultures, the complicated and costly processes of which are known by the experts in the field.

One of the characteristics of the method in accordance with the improvements proposed by the invention is that, retaining the same culture bed, oily fatty residues, specifically residues from crushing olives, known as olive pomace, in accordance with the invention, are subjected to a previous step of sterilization.

Another detail of the invention is that, in a partial variation of the latter, the method contemplates the immunization of the sterilized culture bed.

An essential and vitally important characteristic is that the invention eliminates the use of the typical seeding material; the basidiomycete mycelium obtained in complicated sterile preliminary cultures is replaced by parts or fragments of the whole basidiocarp itself.

In accordance with the method of the invention, it has been possible to find that the basidiomycete obtained in accordance with a sterile culture in the culture bed that the patent proposes and in accordance with the method that will be claimed, is recultured in appropriate portions and is reproduced in the same conditions of yield

and efficiency that the previous patent of the same applicant describes.

The method in accordance with the invention includes a first step of fermentation of the growing material in which the culture bed, the mass of olive pomace, is submerged in water that covers it, within tanks or containers, to be sterilized as much as possible, and preferably in disinfectant media, for a period that varies between 1 and 35 days according to the ambient temperature.

Specifically and preferably the fermentation is carried out within a period that varies between 18 and 35 days in an inverse proportional relation to an ambient temperature of between 25°C and 14°C. Logically and reasonably, the higher the ambient temperature, the lower the fermentation time.

At an ambient temperature of 11°C - 10°C, the fermentation is established.

A second step in which minuscule fragments of basidiomycetes obtained in previous cultures are sowed in layers of humus of the culture bed obtained in the previous step, forming layers approximately 10 cm thick. Each layer is mixed with the fragments, proportionately; it is covered with another layer, seeded with other fragments, and thus until the last layer covers the immediate interior.

The pile of layers forms compact blocks of, preferably, approximately 60 x 25 x 30 cm; associating and combining them with each other, and uniting them differently by the simple rooting of

their vegetative basidia, or in the open air for their natural germination, or in places heated for their winter germination.

The fragments are obtained from the whole mushroom, dividing it arbitrarily into little pieces, that is, the basidiocarp composed of pedicel (stipe), ring (annulus), gills (lamellae), and seeds (pileus) is fractionated.

In a third step, the cultivated beds in accordance with the first step are covered with an envelope of plastic material (thick polyvinyl), except for a zone, preferably centered, that is formed by an opening closed by a piece of textile, a cotton cloth that is breathable, and that prevents the access of elements of contagion.

In a fourth step, the culture or culture block in accordance with the previous step is left to sit and germinate for a period of time of, approximately, six weeks, during which they harden, forming very hard monolithic blocks.

In a fifth step, the envelope is opened, spraying the hardened culture with water for a week and a half, after this period the fruit begins to grow, obtaining a first germination and thus successively until the culture degenerates, ceasing to produce.

The culture may be reused as agricultural fertilizer or fodder for livestock.

A second embodiment of the method in accordance with the invention comprises:

A phase in which the fermented culture of the first step is removed from the water and heated for approximately 60 minutes at the

temperature of boiling water.

Preferably this takes place in sterilized containers and in decontaminated and aseptic surroundings.

For example, in order to avoid costly industrial installations, a small amount may be treated according to the boiling phase mentioned.

The fermented and boiled culture bed, in the form and method in accordance with the invention, first to fifth steps, produces pure, sterilized, aseptic, and decontaminated, basidiomycetes suited for their reproduction or culturing in the form provided and specifically described in the second step of the method.

It is clear from the nature of the invention described that the latter is not limited to the exact details of this description, if modifications are introduced into it that are considered appropriate, provided that they do not alter the essential characteristics of the same that are claimed below.

CLAIMS

1. A method for growing basidiomycetes, that is performed on a culture base composed of olive residue, olive pomace, **wherein**, in a first step, this material is subjected to a fermentation cycle, submerging it in water so as to cover it, in preferably sterilized tanks or containers and aseptic surroundings for a period of time, and at temperatures inversely related to this time, comprising successive steps of culture, germination, harvesting, and/or a second realization of immunization f the culture bed.

2. A method for growing basidiomycetes, in which the step of fermentation, in accordance with Claim 1, **wherein** it is performed in a period of time that varies between 25°C and 14°C, in inverse proportion to the time; the higher the temperature, the fewer days of fermentation.

3. A method for growing basidiomycetes, that comprises a culture base, in accordance with the previous claims, **wherein** in layers composed of the previous culture bed, approximately 10 cm thick, minuscule parts or fragments of the basidiomycetes themselves obtained in pure preliminary cultures or according to the same method of the invention are seeded or cultured.

4. A method for growing basidiomycetes, that comprises a culture base in accordance with the previous claim, **wherein** the operation is repeated by overlapping layers of similar thickness, mixing them respectively, with parts or fragments of the appropriate basidiomycete, until the final one that covers the immediately lower one.

5. A method for growing basidiomycetes, that comprises a growing

base in accordance with Claims 1 and 4, in which the culture beds are **characterized by** forming compact blocks, preferably 60 x 25 x 30 cm, subject to be associated or combined with each other in the germination phase by simple rooting of their vegetative basidia.

6. A method for growing basidiomycetes, that includes compact blocks in accordance with the previous claim, **wherein** they are combined in different forms, in the open air for their natural germination, or in heated places for winter germination.

7. A method for growing basidiomycetes, that includes a culture base in accordance with claims 3 to 6 in which the culture beds are **characterized by** the fact that they are totally covered by an envelope of plastic material, except for a zone, preferably concentric, covered or closed with a textile sheet, preferably a cotton fabric.

8. A method for growing basidiomycetes, that includes a germination phase in accordance with claims 1 to 5, **wherein** it is let to sit for a period of approximately six weeks until the total hardening of the cultured block.

9. A method for growing basidiomycetes, that includes a germination phase in which the hardened bed is **characterized by** the fact that the envelope is opened, spraying the hardened culture with water for a week and a half.

10. A method for growing basidiomycetes that includes a phase of immunization of the culture bed, in accordance with claim 1, in which the residual waste of crushed olives, once fermented, is removed from the water and heated, mixing it with clean water, for 60 minutes at

boiling temperature, in appropriately sterilized containers and decontaminated and aseptic surroundings.

11. A method of growing basidiomycetes.

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Procedure for Producing a Nutrient Medium, especially for the *Lentinus edodes* (Shiitake) Mushroom, and Procedure for Cultivating the *Lentinus edodes* (Shiitake) Mushroom

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APPLICANT	(71):	
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FOREIGN TITLE	(54A):	Eljárás gombatáptalaj, fõként Lentinus edodes (siitake) gomba táptalajának elõállítására és eljárás Lentinus edodes (siitake) gomba termesztésére

(54) Procedure for Producing a Nutrient Medium, especially for the *Lentinus edodes* (Shiitake) Mushroom, and Procedure for Cultivating the *Lentinus edodes* (Shiitake) Mushroom

(57) ABSTRACT

The object of the invention is a procedure for producing a nutrient medium based on lignocellulose, primarily for efficient cultivation of the *Lentinus edodes* (shiitake) mushroom, and a procedure for cultivating the *Lentinus edodes* (shiitake) mushroom.

The invention is, on the one hand, a procedure for producing a mushroom nutrient medium, primarily a nutrient medium for the *Lentinus edodes* (shiitake) mushroom, during which 31-38 wt% air-dried grain chaff and 6-8 wt% dry ground corncobs are mixed together. While continuing to mix the mixture obtained, it is moistened thoroughly with 46-56 wt% water containing 0.09-0.11 wt% nitrogen, phosphorous pentoxide, and potassium oxide as active agents, in which the ratio of nitrogen, phosphorous, and potassium oxide is 1:1:0.75, and 0.002-0.008 wt% 1-butyl-carbamoyl-benzimidazol-2-methyl carbamate in dissolved form. After this, with further mixing, 1.5-3 wt% limestone powder and/or 3-4 wt% gypsum is added to the mixture, which is then heat-treated by introducing water vapor at 59-65 °C. The temperature of the mixture is kept at 56-61 °C for 15 hours, then it is cooled to 22-27 °C.

On the other hand, the invention is a procedure for cultivating *Lentinus edodes* (shiitake) mushrooms is such a way that the nutrient medium prepared according to the above is inoculated in a known manner with 4-8 wt% *Lentinus edodes* mycelium spawn, texturized for 15-25 days at 22-28 °C, then cultivated at 12-18 °C for 85-95 days.

The object of the invention is a procedure for producing a lignocellulose nutrient medium serving for efficient cultivation of mushrooms, primarily *Lentinus edodes* (shiitake) mushrooms.

Numerous procedures for cultivating large species of edible mushrooms have become known. One of the most widely used methods was disclosed in Hungarian patent description number 191675, which related to preparation of a nutrient medium suitable for the

production of mushrooms, primarily the oyster mushroom. Procedures serving for the cultivation of *Pleurotus* (oyster mushrooms) and *Agaricus* (champignon mushrooms) have also become known. We can mention one such procedure from Hungarian patent description number 197482, which relates to the cultivation of champignon species, primarily *Agaricus bisporus*, *Agaricus bitorquis*, and *Agaricus macrosporoides*. Also known is the procedure published in Hungarian patent description number 187502, which relates to the intensive cultivation of mushrooms not requiring a fertilizer, primarily *Pleurotus* species (oyster mushrooms).

The procedures mentioned above that are part of the state of the art all have the common deficiency that they are not at all applicable to the cultivation of the *Lentinus edodes* mushroom, because the compositions of the nutrient media and the spectrums of the various oligo- and polysaccharides are very unfavorable for *Lentinus edodes:* the growth of the mycelia is exceptionally slow and weak and competing microorganisms appear in large masses that destroy the mycelium of the shiitake mushroom introduced at the time of inoculation. Methods based on the wood material that forms the source of nutrients used the areas where the shiitake mushroom grows naturally (primarily Southeast Asia) cannot be used economically in other geographical areas because of the limited availability of the wood types and the texturizing, which requires years to become productive.

The goal of the invention is, on the one hand, to create a new procedure that will make it possible to produce a nutrient medium that is suitable for cultivation of the large shiitake edible mushroom (*Lentinus edodes*) and, on the other, to cultivate the large shiitake edible mushroom (*Lentinus edodes*) on the nutrient medium produced. Within this, a task of the invention is that the nutrient medium will have selectivity such that rapid and strong growth of the shiitake mycelium is made possible but reproduction of competing microorganisms (mildews, shaggy-cap mushrooms, etc.).

The invention is based on the recognition of if air-dried grain chaff are mixed in a desired proportion with ground corncobs, then the water content of the mixture obtained is set to a given value and limestone powders and/or gypsum is added to the mixture along with 1-butyl-carbamoyl-benzimidazol-2-methyl carbamate, then an artificial nutrient medium is obtained for the shiitake mushroom species with which it becomes possible to cultivate this mushroom, which has not previously been cultivated artificially.

Thus our invention is, on the one hand, a procedure for producing a mushroom medium, especially a nutrient medium for *Lentinus edodes* (shiitake), during which grain chaff and ground corncobs are mixed together. According to the invention, we proceed by mixing together 31-38 wt% air-dried grain straw and 6-8 wt% ground dry corncobs, and, while continuing to mix the mixture, moistening it thoroughly with 46-56 wt% water containing 0.09-0.11 wt% nitrogen,

phosphorous pentoxide, and potassium oxide as active agents, in which the ratio of nitrogen, phosphorous, and potassium oxide is 1:1:0.75, and 0.002-0.008 wt% 1-butyl-carbamoyl-benzimidazol-2-methyl carbamate in dissolved form, after which, with further mixing, 1.5-3 wt% limestone powder and/or 3-4 wt% gypsum is added to the mixture, which is then heat-treated by introducing water vapor at 59-65 °C. The temperature of the mixture is kept at 56-61 °C for 15 hours, then it is cooled to 22-27 °C.

According to an advantageous embodiment, wheat-straw chaff is formed with a 20-mm sieve equipped with a hammer mill.

On the other hand, our invention is a procedure for cultivating *Lentinus edodes* (shiitake) in such a way that the nutrient medium produced according to the above is inoculated in a known manner with 4-8 wt% *Lentinus edodes* mycelium spawn (particle size: 10^4-10^6 per gram), then it is texturized for 15-25 days at 22-28 °C, and cultivated at 12-18 °C for 85-95 days.

Advantageously, the prepared nutrient medium is placed in polyethylene bags with diameter 35-45 cm and the texturizing and then the cultivation are continued thereafter in these bags.

In the following, the invention will be illustrated with recipes and examples taken from the recipes.

Example 1	*kg*	*% by weight (wt%)*
air-dried wheat-straw chaff	6,870	34.3
ground corncobs	1,340	6.7
water	10,279.4	51.4
inorganic artificial fertilizer (nitrogen, phosphorus pentoxide, potassium oxide, ratio 1:1:0.75)	20	0.1
1-butyl-carbamoyl-benzimidazol 2-methyl carbamate	0.6	0.003
limestone powder	420	2.1
Lentinus edodes mycelium spawn	1,070	5.4
nutrient medium to fill one chamber	*20,000*	*100*

34.3 wt% air-dried wheat straw cut to 5-cm chaff is mixed with 6.7 wt% corncobs, likewise air-dried, and ground in a hammer mill equipped with a 20-mm sieve. This mixture is moistened evenly with continual stirring with 51.4% of a moistening liquid that is produced by dissolving an inorganic artificial fertilizer in which the ratio of nitrogen phosphorus pentoxide and potassium oxide is 1:1:0.75 (commercial preparation called Wuxal) and 0.003 wt% 1-butyl-carbamoyl-benzimidazol-2-methyl carabamate as active agent (commercial preparation called Fundazol 50 WP).

To the materials moistened in this way, 2.1 wt% limestone powder is added, then, after a liquefaction period of 2 hours, this material is placed in a heat-treatment chamber. The stored material is heated to 62 °C by blowing in air and hot steam intensively. The temperature reached is maintained at 59 °C, then the material is cooled spontaneously to 25 °C and after it is removed from the chamber, 5.4 wt% *Lentinus edodes* mycelium spawn is mixed into it, which contains

10^5 cells per gram. The medium produced in this manner is placed in perforated polyethylene bags with a diameter of 40 cm and a capacity of 10 kg, then texturized for 20 days at a temperature of 25 °C. After this, the temperature in the cultivation area is lowered to 15 °C and the production bodies that appear are collected over a period of 90 days.

Example 2	*kg*	*% by weight (wt%)*
air-dried wheat-straw chaff	4,900	24.5
ground corncobs	1,600	8
water	11,499.2	57.5
inorganic artificial fertilizer (nitrogen, phosphorus pentoxide, potassium oxide, ratio 1:1:0.75)	20	0.1
1-butyl-carbamoyl-benzimidazol 2-methyl carbamate	0.8	0.004
gypsum	700	3.5
Lentinus edodes mycelium spawn	1,280	6.4
nutrient medium to fill one chamber	*20,000*	*100*

Proceed in every way according to Example 1, but the 0.1% inorganic fertilizer is added to the moistening liquid and the 3.5 wt% gypsum is added after the moistening.

The most important advantage of the procedure according to the invention is that it makes cultivation possible of a very valuable mushroom on an artificial nutrient medium and accelerates the turnaround time for the product (in nature, this requires several years).

PATENT CLAIMS

1. A procedure for producing a mushroom nutrient medium, especially nutrient medium for *Lentinus edodes* (shiitake) using

materials containing cellulose, *characterized by the fact* that, with respect to total weight the mushroom nutrient medium, 31-38 wt% air-dried grain chaff and 6-8 wt% dry ground corncobs are mixed together, the mixture obtained is moistened while being fixed further with water containing 0.09-0.11 wt% nitrogen, phosphorus pentoxide, and potassium oxide as active agents, in which the ratio of nitrogen, phosphorus pentoxide, and potassium oxide is 1:1:0.75, and 0.002-0.008 wt% 1-burtyl-carbamoyl-benizimidazol-2-methyl carbamate in dissolved form, and with further mixing, 1.5-3 wt% limestone powder and/or 3-4 wt% gypsum is added to the mixture, the temperature of the mixture is kept at 59-65 °C for 15 hours, then it is cooled to 22-27 °C.

2. A procedure according to Claim 1, *characterized by the fact* that wheat-straw chaff is used as the grain chaff.

3. A procedure according to Claim 1 or 3, *characterized by the fact* that the wheat-straw chaff is formed in a hammer mill equipped with a 20-mm sieve.

4. A procedure for cultivating *Lentinus edodes* (shiitake) mushrooms, *characterized by the fact* that nutrient medium produced according to claim 1 is inoculated with 4-8% *Lentinus edodes* mycelium in a known manner, where the number of cells is 10^4-10^5 per gram, then texturized for 15-25 days at 22-28 °C and cultivated at 12-18 °C for 85-95 days.

5. A procedure according to Claim 4, *characterized by the fact* that the nutrient medium is placed in polyethylene bags with a diameter of 35-45 cm and the texturizing and cultivation are continued in these bags.